DNA REPAIR

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Reference texts: Berg et al, Chapter 28
Weaver, 5th ed, Chapters 20 and 22
Friedberg, 2nd ed, DNA Repair and Mutagenesis

DNA Repair

Mutations and DNA damage
- -agents that cause DNA damage

DNA repair pathways
- -direct repair
- -excision repair
- -mismatch repair
- -double strand break repair

Cellular responses to DNA damage
- -SOS response in E.coli
- -checkpoint response in eukaryotic cells

DNA damage ≠ Mutation

DNA damage not repaired → Mutation

Some DNA Damage Is Due to DNA Replication Errors

A Variety of Agents Cause DNA Damage

A. Some Bases Can Be Deaminated

B. Base Analogs Can Be Incorporated into DNA During Replication

C. Some Alkylating Agents Can Damage DNA

D. Intercalating Agents Can Cause Insertions and Deletions

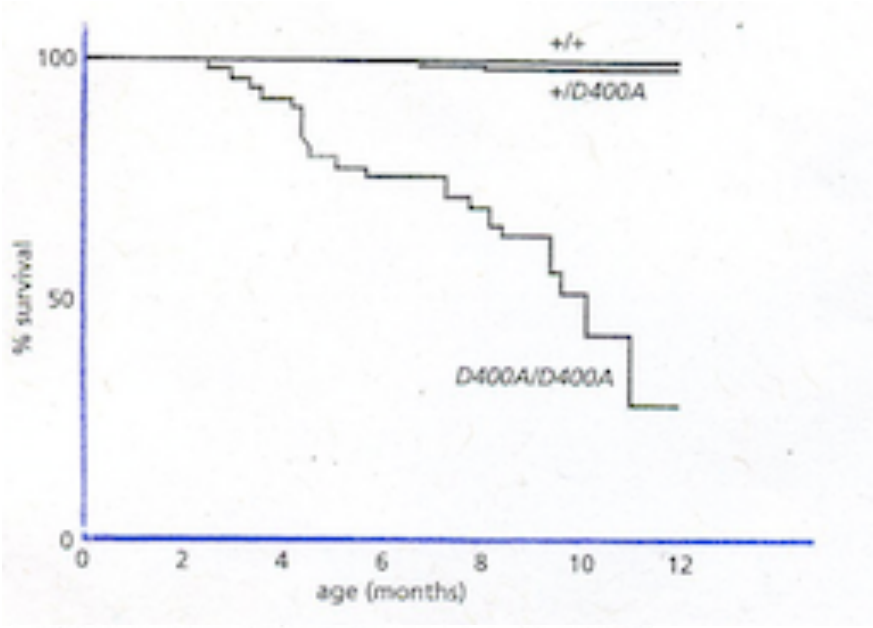
E. Radiation Damages DNA

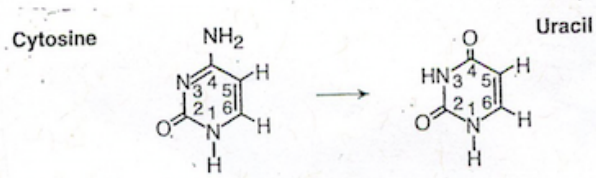
Table 1–1 Mechanisms for maintaining genetic stability associated with DNA replication in *E. coli*

Mechanism	Cumulative error frequency
Base pairing	$\sim 10^{-1}$–10^{-2}
DNA polymerase actions (including base selection and 3′ → 5′ proofreading exonuclease)	$\sim 10^{-5}$–10^{-6}
Accessory proteins (e.g., single-strand binding protein)	$\sim 10^{-7}$
Postreplicative mismatch correction	$\sim 10^{-10}$

(From Friedberg)

Figure 12.7 Proofreading by DNA polymerase and cancer incidence
A point mutation has been introduced into the germ-line copy of the mouse gene encoding DNA polymerase δ, the mammalian DNA polymerase that is responsible for the bulk of leading and lagging strand synthesis. This mutation, termed *D400A*, alters the amino acid sequence in the proofreading domain of the polymerase by specifying the replacement of an aspartic acid by an alanine at residue position 400 of the polymerase molecule. Shown here is the fate of 53 wild-type mice (+/+), 97 heterozygotes (*+/D400A*), and 49 homozygous mutants (*D400A/D400A*). Deaths of the mutant homozygotes were all due to malignancies; these included lymphomas, squamous cell carcinomas of the skin, and several other types of cancer that occurred relatively infrequently. Two of the heterozygotes died from causes that were unrelated to cancer, while the homozygous wild-type mice all survived to the age of one year. Their survival curves are shown here in this Kaplan–Meier plot. (From R.E. Goldsby, N.A. Lawrence, L.E. Hays et al., *Nat. Med.* 7:638–639, 2001.)





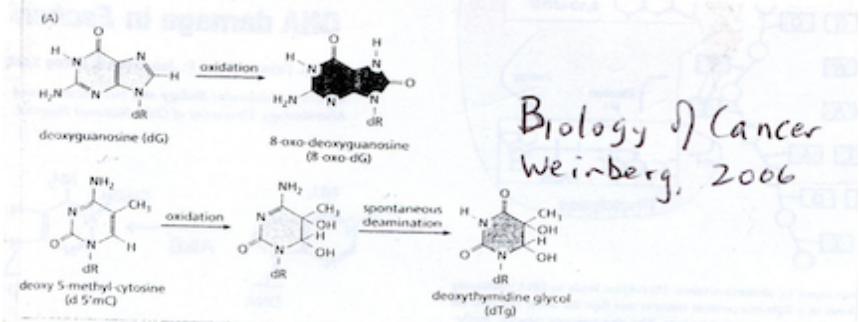
Cell genomes are under constant attack from endogenous biochemical processes

The intracellular environment holds yet other dangers for the chromosomal DNA. The greatest of these comes from the processes of oxidation, which may inflict far more damage on DNA than the reactions mentioned above. Most important here are the reactions that occur in the mitochondria and generate a variety of intermediates as oxygen is progressively reduced to water:

$$O_2 + e^- \rightarrow O_2^{\bullet-} + e^- \rightarrow H_2O_2 + e^- \rightarrow {}^{\bullet}OH + e^- \rightarrow H_2O$$

superoxide ion — hydrogen peroxide — hydroxyl radical

Some of these intermediates, the so-called reactive oxygen species (ROS), may leak out of the mitochondria into the cytosol and thence into the rest of the cell. Included among these are the superoxide ion, hydrogen peroxide, and the hydroxyl radical—the intermediates in the reactions listed above.



Biology of Cancer
Weinberg, 2006

Figure 12.12 **Oxidation of bases in the DNA.** The oxidation of DNA bases, which often results from the actions of reactive oxygen species (ROS), can be mutagenic in the absence of subsequent DNA repair reactions. (A) Two frequent oxidation reactions involve deoxyguanosine (dG), which is oxidized to 8-oxo-deoxyguanosine (8-oxo-dG), and deoxy-5-methylcytosine (d-5'-mC), the nucleotide that is present in methylated CpG sequences. Upon oxidation, the latter initially forms an unstable base that rapidly deaminates, yielding deoxythymidine glycol (dTg). (B) The 8-oxo-dG, which is formed by the oxidation of dG, can mispair with deoxyadenosine (dA) rather than forming a normal base pair with deoxycytosine (dC). Hence, if 8-oxo-dG is not removed from a double helix, the DNA replication machinery may inappropriately incorporate a dA rather than a dC opposite it, resulting in a C→A point mutation.

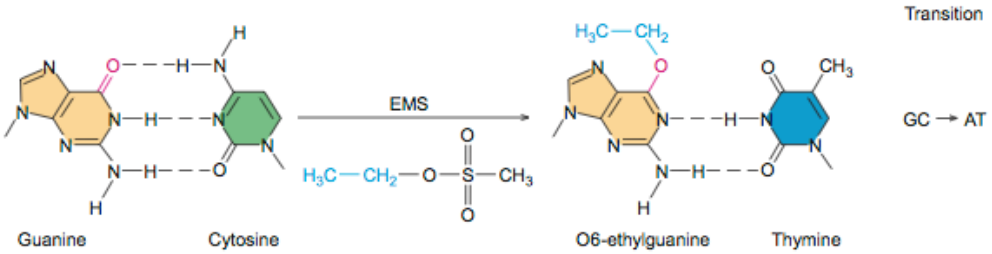


Figure 20.25 Alkylation of guanine by EMS. At the left is a normal guanine–cytosine base pair. Note the free O6 oxygen (red) on the guanine. Ethylmethane sulfonate (EMS) donates an ethyl group (blue) to the O6 oxygen, creating O6-ethylguanine (right), which base-pairs with thymine instead of cystosine. After one more round of replication, an A–T base pair will have replaced a G–C pair.

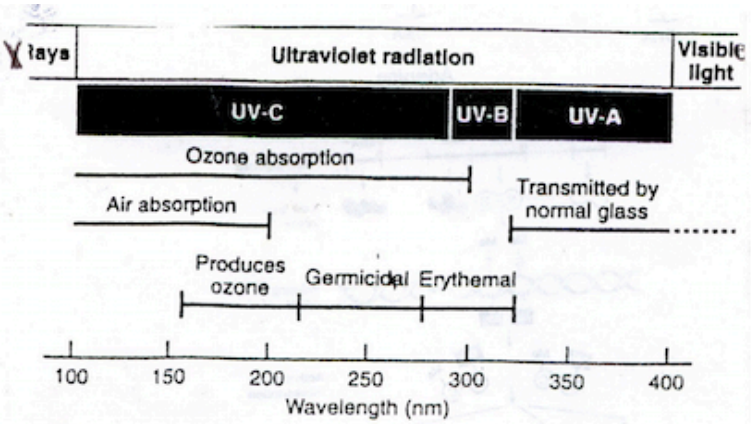
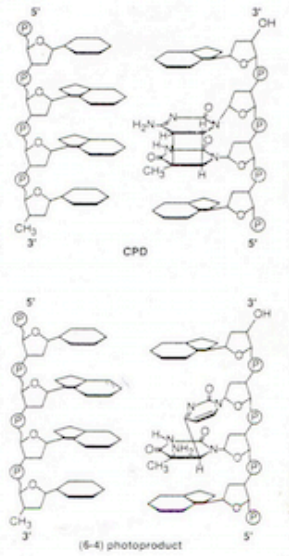


Figure 2-19 UV radiation spectrum. Friedberg

UV - photoproducts



PR was the first DNA repair mode to be discovered. In the late 1940s Albert Kelner, then at the Cold Spring Harbor Laboratory, was studying the effects of UV radiation on certain strains of the fungus *Streptomyces griseus* (102). While investigating the influence of postirradiation temperature on colony survival, Kelner was plagued by an experimental variable, the systematic exploration of which ultimately led to the recognition of a specific role of light in the recovery of UV-irradiated cells. Kelner's own description of this discovery merits quotation (102):

> Careful consideration was made of variable factors which might have accounted for such tremendous variation. We were using a glass-fronted water bath placed on a table near a window, in which were suspended transparent bottles containing the irradiated spores. The fact that some of the bottles were more directly exposed to light than others suggested that light might be a factor. Moreover, the greatest and most consistent recovery in our preliminary experiments had taken place in suspensions stored in transparent bottles at room temperature on an open shelf exposed to diffuse light from a window. Experiment showed that exposure of ultra-violet irradiated suspensions to light resulted in an increase in survival rate or a recovery of 100,000- to 400,000-fold. Controls kept in the dark . . . showed no recovery at all. The magnitude of the light effect can hardly be overemphasized. The recovery was so much more complete than any previously observed, that we felt we were dealing here with a key factor in the mechanism causing inactivation and recovery from ultra-violet irradiation.

At about the same time, Renato Dulbecco, then at the Department of Bacteriology, Indiana University, reported the same phenomenon in the T group of coliphages (39). He wrote that:

> the occurrence of photo-reactivation of ultra-violet irradiated phage was noticed accidentally a few weeks after receiving a personal communication from Dr. A. Kelner that he had discovered recovery of ultra-violet treated spores of *Actinomycetes* upon exposure to visible light. I am informed by Dr. Kelner that his results are in course of publication. My observation indicates the correctness of Dr. Kelner's suggestion that the phenomenon discovered by him may be of general occurrence for a number of biological objects.

Friedberg

Table 1–1 Biological responses to DNA damage

Reversal of base damage
Excision of damaged, mispaired, or incorrect bases
Base excision repair (BER)
Nucleotide excision repair (NER)
Transcription-coupled nucleotide excision repair (TC-NER)
Alternative excision repair (AER)
Mismatch repair (MMR)
Strand break repair
Single-strand break repair (SSBR)
Double-strand break repair (DSBR)
Tolerance of base damage
Translesion DNA synthesis (TLS)
Postreplicative gap filling
Replication fork progression
Cell cycle checkpoint activation
Apoptosis

Base Excision Repair

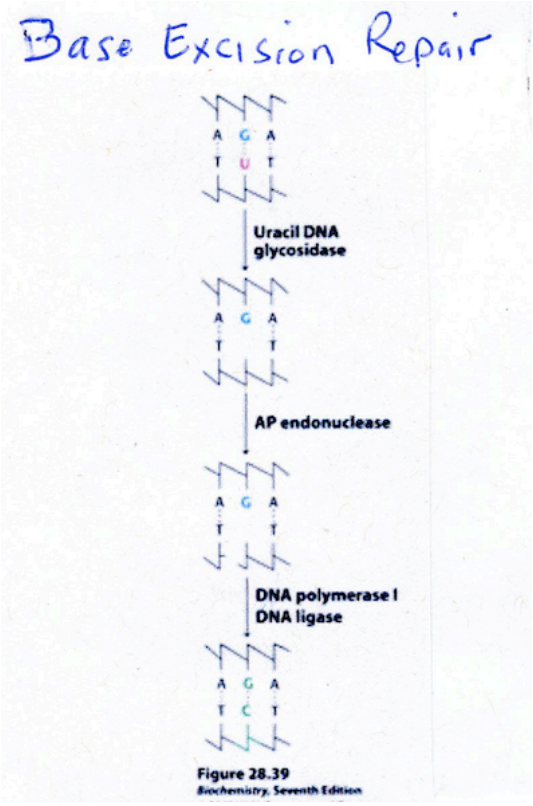


Figure 28.39
Biochemistry, Seventh Edition

Figure 28.39 Uracil repair. Uridine bases in DNA, formed by the deamination of cytidine, are excised and replaced by cytidine.

Table 4–2 DNA glycosylases

Enzyme	Substrate	Products
Ura-DNA glycosylase	DNA containing uracil	Uracil + AP sites
Hmu-DNA glycosylase	DNA containing hydroxymethyluracil	Hydroxymethyluracil + AP sites
5-mC-DNA glycosylase	DNA containing 5-methylcytosine	5-methylcytosine + AP sites
Hx-DNA glycosylase	DNA containing hypoxanthine	Hypoxanthine + AP sites
Thymine mismatch-DNA glycosylase	DNA containing G·T mispairs	Thymine + AP sites
MutY-DNA glycosylase	DNA containing G·A mispairs	Adenine + AP sites
3-mA-DNA glycosylase I	DNA containing 3-methyladenine	3-Methyladenine + AP sites
3-mA-DNA glycosylase II	DNA containing 3-methyladenine,7-methylguanine, or 3-methylguanine	3-Methyladenine, 7-methylguanine, or 3-methylguanine + AP sites
FaPy-DNA glycosylase	DNA containing formamidopyrimidine moieties, or 8-hydroxyguanine	2,6-Diamino-4-hydroxy-5-*N*-methylformamido-pyrimidine and 8-hydroxyguanine + AP sites
5,6-HT-DNA glycosylase (endonuclease III)	DNA containing 5,6-hydrated thymine moieties	5,6-Dihydroxydi-hydrothymine or 5,6-dihydrothymine + AP sites
PD-DNA glycosylase	DNA containing pyrimidine dimers	Pyrimidine dimers in DNA with hydrolyzed 5′ glycosyl bonds + AP sites

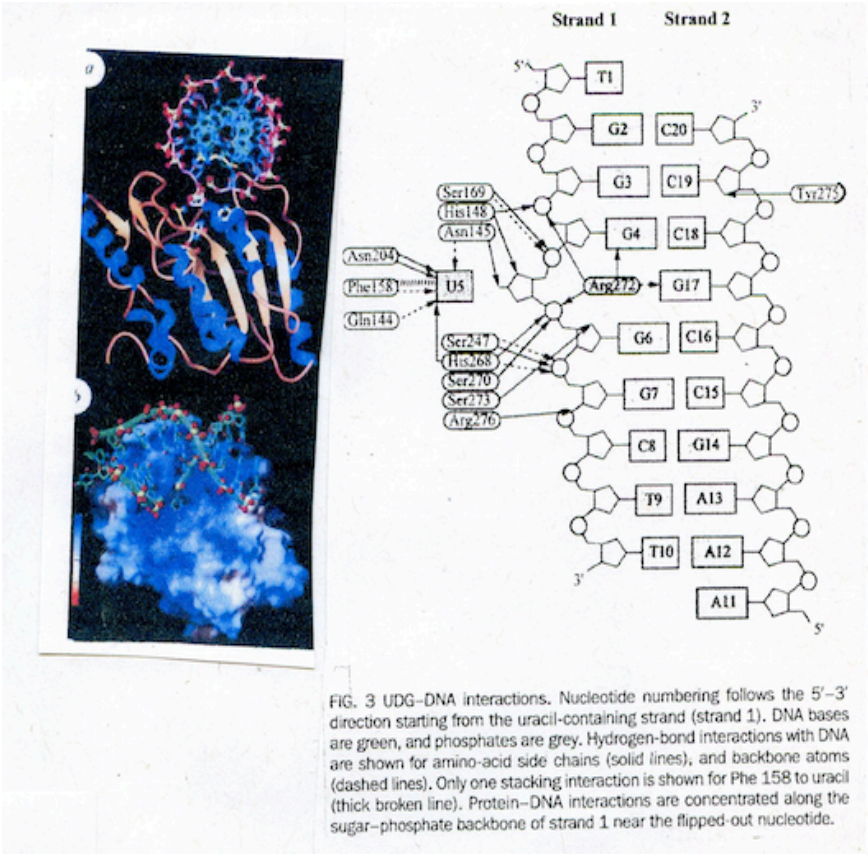


FIG. 3 UDG–DNA interactions. Nucleotide numbering follows the 5′–3′ direction starting from the uracil-containing strand (strand 1). DNA bases are green, and phosphates are grey. Hydrogen-bond interactions with DNA are shown for amino-acid side chains (solid lines), and backbone atoms (dashed lines). Only one stacking interaction is shown for Phe 158 to uracil (thick broken line). Protein–DNA interactions are concentrated along the sugar–phosphate backbone of strand 1 near the flipped-out nucleotide.

A nucleotide-flipping mechanism from the structure of human uracil–DNA glycosylase bound to DNA

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Any uracil bases in DNA, a result of either misincorporation or deamination of cytosine, are removed by uracil-DNA glycosylase (UDG), one of the most efficient and specific of the base-excision DNA-repair enzymes[1]. Crystal structures of human[2,3] and viral[4] UDGs complexed with free uracil have indicated that the enzyme binds an extrahelical uracil. Such binding of undamaged extrahelical bases has been seen in the structures of two bacterial methyltransferases[5,6] and bacteriophage T4 endonuclease V (ref. 7). Here we characterize the DNA binding and kinetics of several engineered human UDG mutants and present the crystal structure of one of these, which to our knowledge represents the first structure of any eukaryotic DNA repair enzyme in complex with its damaged, target DNA. Electrostatic orientation along the UDG active site, insertion of an amino acid (residue 272) into the DNA through the minor groove, and compression of the DNA backbone flanking the uracil all result in the flipping-out of the damaged base from the DNA major groove, allowing specific recognition of its phosphate, deoxyribose and uracil moieties. Our structure thus provides a view of a productive complex specific for cleavage of uracil from DNA and also reveals the basis for the enzyme-assisted nucleotide flipping by this critical DNA-repair enzyme.

UDG belongs to the base-excision repair pathway that repairs the more than 10,000 bases damaged daily in each human cell[8]. The most common forms of DNA damage occur by oxidation, depurination, alkylation and deamination. Cytosine deamination alone amounts to 100–500 uracil bases per cell per day[9].

from Nature 384, 87, 1996

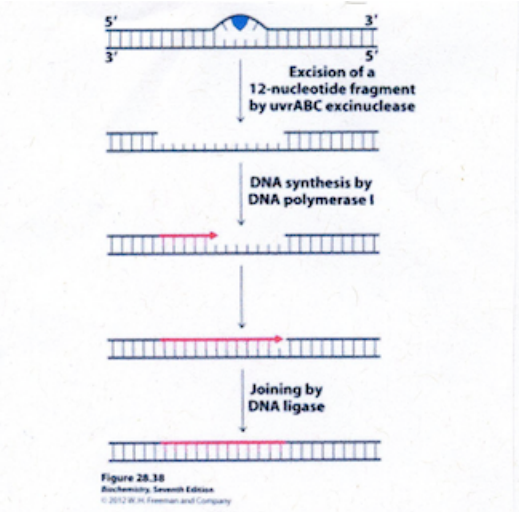


Figure 28.38 Nucleotide-excision repair. Repair of a region of DNA containing a thymine dimer by the sequential action of a specific exinuclease, a DNA polymerase, and a DNA ligase. The thymine dimer is shown in blue and the new region of DNA is in red.

Table 7.2 Genes affected in XP patients, and encoded proteins

Human Gene	Protein Function	Homologous to *S. cerevisiae*	Analogous t *E. coli*
XPA	Binds damaged DNA	Rad14	UvrA/UvrB
XPB	3' to 5' helicase, component of TFIIH	Rad25	UvrD
XPC	DNA-damage sensor (in complex with hHR23B)	Rad4	
XPD	5' to 3' helicase, component of TFIIH	Rad3	UvrD
XPE	Binds damaged DNA		UvrA/UvrB
XPF	Works with ERRC1 to cut DNA on 5' side of damage	Rad1	UvrB/UvrC
XPG	Cuts DNA on 3' side of damage	Rad2	UvrB/UvrC

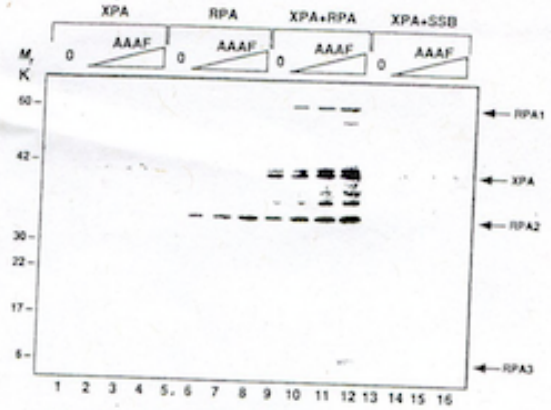


FIG. 3 RPA and XPA bind AAAF-damaged DNA cooperatively. Cooperative binding of damaged DNA. Biotinylated DNA fragments were untreated (lanes 1, 5, 9, 13), or treated with 5 mM (lanes 2, 6, 10, 14), 10 mM (lanes 3, 7, 11, 15), or 50 mM (lanes 4, 8, 12, 16) AAAF, bound to streptavidin-beads and incubated at 4 °C for 2 h with 0.5 μg of XPA (lanes 1–4), 1.0 μg of RPA (lanes 5–8), 0.5 μg of XPA and 1.0 μg of RPA (lanes 9–12), or 0.5 μg of XPA and 0.5 μg of *E. coli* SSB (USB, lanes 13–16). The DNA-bound XPA and RPA were similarly analysed with a mixture of anti-RPA and anti-XPA sera.

He et al, Nature 374, 566, 1995

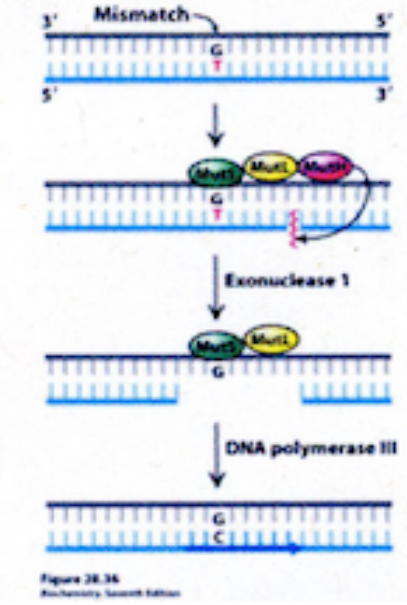
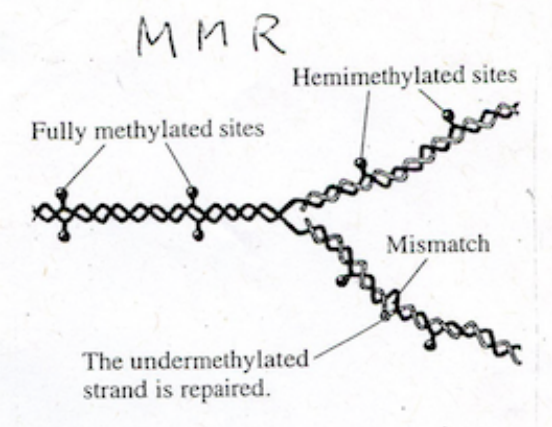


Figure 28.36 Mismatch repair. DNA mismatch repair in *E. coli* is initiated by the interplay of MutS, MutL, and MutH proteins. A G–T mismatch is recognized by MutS. MutH cleaves the backbone in the vicinity of the mismatch. A segment of the DNA strand containing the erroneous T is removed by exonuclease I and synthesized anew by DNA polymerase III. [After R. F. Service. *Science* 263:1559–1560, 1994.]



Mismatch repair of methylated DNA. Immediately after replication, newly synthesized DNA (light gray) is undermethylated. A mismatch-repair mechanism recognizes the new DNA and selectively repairs the newly synthesized strand using the parental strand as a template. (Methylated bases are identified by orange dots.)

DNA is damaged by double-strand breaks

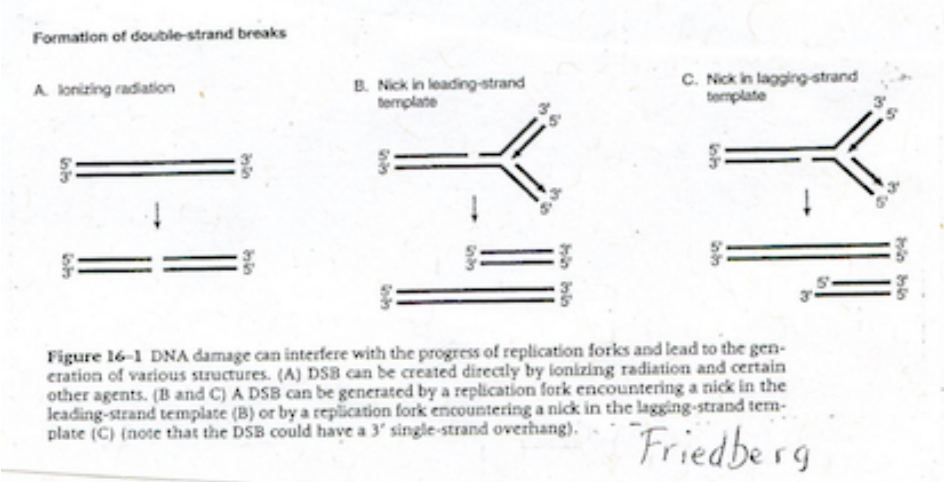


Figure 16–1 DNA damage can interfere with the progress of replication forks and lead to the generation of various structures. (A) DSB can be created directly by ionizing radiation and certain other agents. (B and C) A DSB can be generated by a replication fork encountering a nick in the leading-strand template (B) or by a replication fork encountering a nick in the lagging-strand template (C) (note that the DSB could have a 3′ single-strand overhang). Friedberg

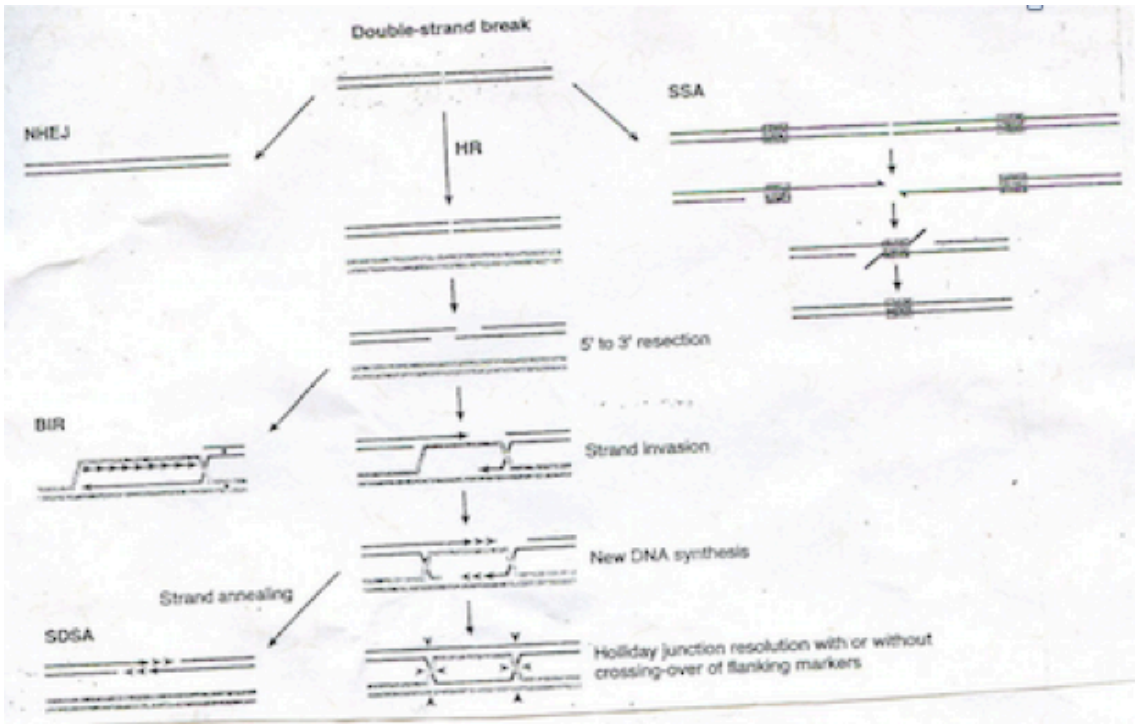


Figure 18–1 Overview of eukaryotic DSB repair pathways. A DNA DSB can be resealed by NHEJ without the participation of a homologous partner (see chapter 19 for details). The alternative pathways are initiated by 5′ → 3′ single-strand resection. Strand invasion can result in the formation of a replication fork and an extended gene conversion tract (BIR). Following limited DNA synthesis, using another homologous chromosome or chromatid as a template, the invading strands may reanneal, and no crossover takes place (SDSA). However, in the classical HR scheme, Holliday junctions are resolved through symmetrical scissions following branch migration, resulting in gene conversion tracts in the vicinity of the DSB and possibly the crossover of flanking markers. Lastly, SSA refers to a process whereby single-strand degradation results in the exposure of homologous regions close to the DSB, usually on the same DNA molecule. The process results in annealing, single-strand clipping, and deletion of the intervening sequence.

DAMAGE TOLERATED

Figure 15–1 Translesion synthesis. A polymerase incorporates an incorrect nucleotide opposite a noninstructional or misinstructive lesion (represented by the gold circle) and then continues on.

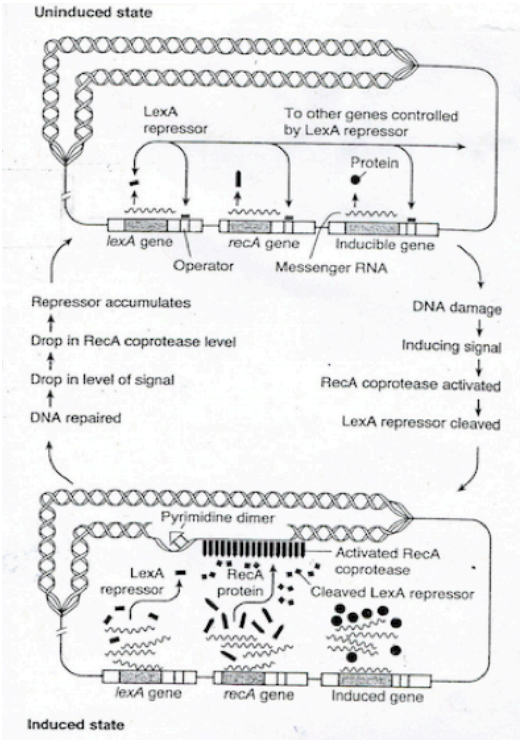


Figure 14–1 Diagrammatic representation of the mechanism by which the *lexA-recA* regulon is regulated. In the uninduced state (top), LexA repressor protein constitutively expressed in small amounts is bound to the *lexA* operator and to the operators of the *recA* gene and other genes under LexA control. These genes are still able to express small amounts of the proteins they encode; thus, there is some RecA protein constitutively present in uninduced cells. Following DNA damage (e.g., the presence of a pyrimidine dimer near a replication fork after induction by UV radiation), the coprotease activity of existing RecA protein is activated, probably by binding to the ssDNA in the gaps created by discontinuous DNA synthesis past the dimers (bottom). The interaction between LexA and activated RecA results in the proteolytic cleavage of LexA. In the induced state (bottom), derepression of the $recA^+$ gene results in the production of large amounts of RecA protein. Other genes under LexA control are also derepressed, although not necessarily with identical kinetics. When the inducing signal disappears (probably by repair of the single-strand gap), the level of active coprotease drops, LexA repressor accumulates, and genes under LexA control are once again repressed.

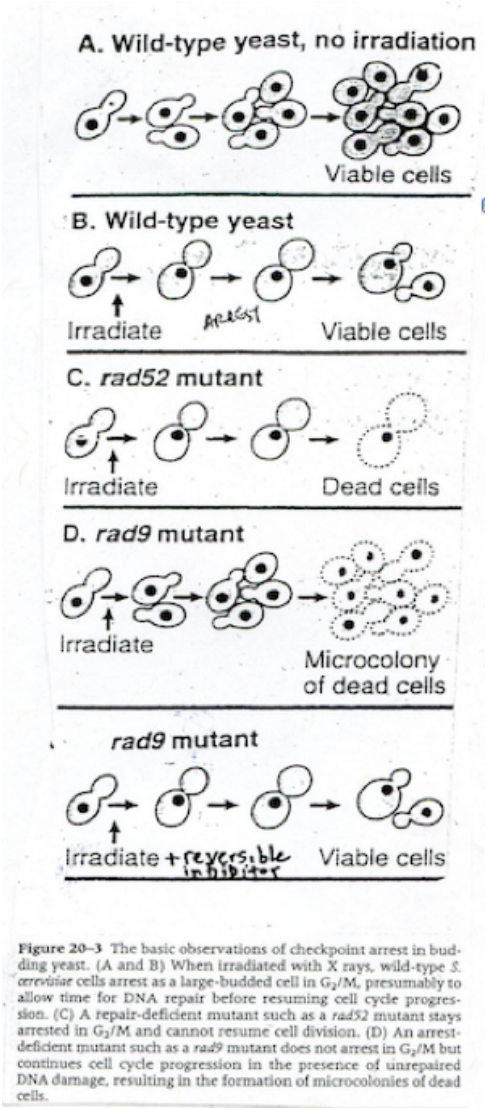


Figure 20–3 The basic observations of checkpoint arrest in budding yeast. (A and B) When irradiated with X rays, wild-type *S. cerevisiae* cells arrest as a large-budded cell in G_2/M, presumably to allow time for DNA repair before resuming cell cycle progression. (C) A repair-deficient mutant such as a *rad52* mutant stays arrested in G_2/M and cannot resume cell division. (D) An arrest-deficient mutant such as a *rad9* mutant does not arrest in G_2/M but continues cell cycle progression in the presence of unrepaired DNA damage, resulting in the formation of microcolonies of dead cells.